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Urinary Biopyrrins Levels Are Elevated in Relation to Severity of Heart Failure

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OBJECTIVES We investigated the relationship between the urinary levels of biopyrrins and the severity of heart failure (HF).

BACKGROUND Oxidative stress is evident in heart disease and contributes to the development of ventricular dysfunction in patients with HF. Biopyrrins, oxidative metabolites of bilirubin, have been discovered as potential markers of oxidative stress.

METHODS We measured the levels of urinary biopyrrins and plasma B-type natriuretic peptide (BNP) in 94 patients with HF (59 men; mean age 65 years) and 47 control subjects (30 men; mean age 65 years). Urine and blood samples were taken after admission in all subjects. Further urine samples were obtained from 40 patients after treatment of HF.

RESULTS The urinary biopyrrins/creatinine levels (μmol/g creatinine) were the highest in patients in New York Heart Association (NYHA) class III/IV (n = 26; 17.05 [range 7.85 to 42.91]). The urinary biopyrrins/creatinine levels in patients in NYHA class I (n = 35; 3.46 [range 2.60 to 5.42]) or II (n = 33; 5.39 [range 3.37 to 9.36]) were significantly higher than those in controls (2.38 [range 1.57 to 3.15]). There were significant differences in urinary biopyrrins/creatinine levels among each group. The treatment of HF significantly decreased both urinary biopyrrins/creatinine levels (from 7.43 [range 3.84 to 17.05] to 3.07 [range 2.21 to 5.71]) and NYHA class (from 2.5 ± 0.1 to 1.7 ± 0.1). Log biopyrrins/creatinine levels were positively correlated with log BNP levels ($r = 0.650$, $p < 0.001$).

CONCLUSIONS These results indicate that urinary biopyrrins levels are increased in patients with HF and are elevated in proportion to its severity. (J Am Coll Cardiol 2004;43:1880–5) © 2004 by the American College of Cardiology Foundation

Congestive heart failure (HF) is a major cause of morbidity and mortality (1). Both acute and chronic HF has been reported to be associated with increased oxidative stress and reduced antioxidant reserve (2). Indeed, reactive oxygen species (ROS) have been reported to be involved in both the genesis and progression of HF (3–6).

Bilirubin may be harmful in vivo, as marked elevation of serum levels can result in the neuronal injury associated with kernicterus. Recently, however, the antioxidative action of bilirubin has been clarified in vitro, and bilirubin is now considered to be an important scavenger of ROS in vivo (7–9). Oxidative metabolites of bilirubin have been identified in human urine and plasma using an antibilirubin monoclonal antibody (10). These metabolites were designated as biopyrrins and are considered to represent potential markers of oxidative stress (11). Recently, we have reported on the relationship between the prognosis of acute myocardial infarction and oxidative stress using urinary biopyrrins levels (12).

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We and others have shown that B-type natriuretic peptide (BNP) is predominantly secreted and released from the ventricles in proportion to the severity of left ventricular dysfunction in patients with HF, and there is increasing evidence that BNP is a sensitive marker of the severity of HF (13–16).

We postulated that urinary biopyrrins may be used as a marker of oxidative stress in vivo and may be a useful marker of the severity of HF. Therefore, in the present study, we examined the relationship between urinary biopyrrins levels, the severity of HF by New York Heart Association (NYHA) functional class, and the plasma levels of BNP.

METHODS

Study population. The study population consisted of 94 consecutive patients with heart disease (59 men and 35 women; mean age 65 ± 1 year). The diagnosis of heart disease was based on the patient's clinical history, physical examination, electrocardiogram (ECG), chest X-ray, echocardiogram, left ventriculogram, and coronary angiogram. Patients in NYHA functional class I had cardiac disease that did not limit physical activity, such that ordinary physical activity did not cause undue fatigue, palpitation, dyspnea, or anginal pain. The remaining patients with heart disease were symptomatic for HF. Patients in NYHA functional class III/IV had significant clinical findings (e.g., edema, S_3,

Abbreviations and Acronyms

BNP = B-type natriuretic peptide
HF = heart failure
LVEF = left ventricular ejection fraction
NYHA = New York Heart Association
ROS = reactive oxygen species

pulmonary congestion). The cause of heart disease was idiopathic dilated cardiomyopathy in 18 patients, hypertrophic cardiomyopathy in 9, old myocardial infarction in 37, hypertensive heart disease in 12, valvular heart disease in 16, and congenital heart disease in 2. Patients with malignant disease, severe lung disease, severe renal failure, and severe liver dysfunction were excluded. The NYHA functional classification was evaluated at the time of admission. Left ventricular ejection fraction (LVEF) was also measured by Simpson's rule, using echocardiography.

We also included 47 controls (30 men and 17 women; mean age 65 ± 2 years), who were age-matched with the patients with heart disease. All of the controls underwent diagnostic cardiac catheterization because they had a history of chest pain with multiple risk factors or an ECG abnormality. However, their coronary angiograms revealed no evidence of coronary stenosis or coronary spasm after intracoronary injection of acetylcholine. Written, informed consent was obtained from all subjects before the study. The study was in agreement with the guidelines approved by the Ethics Committee at our institution.

Urinary biopyrrins assays. In all subjects, urine samples for biopyrrins were taken immediately after admission. Of 59 patients with HF (NYHA classes II and III/IV), 40 patients underwent repeat urinary sampling and a re-evaluation of the NYHA class at the time of discharge. The remaining 19 patients required percutaneous coronary interventions or operation or developed complications such as pneumonia, cerebral infarction, and deterioration of renal function. According to clinical indications, all 40 patients were treated with varying combinations of angiotensin-converting enzyme inhibitors or angiotensin II type 1 receptor blockers (78%), diuretics (80%), beta-blockers (40%), digitalis (20%), and vasodilators (50%). All urine samples were immediately stored at −80°C and protected from light until analyzed.

Urinary biopyrrins were measured in duplicate, using a biopyrrins enzyme immuno assay kit based on monoclonal antibody (Shino-test Co., Tokyo, Japan) (10,12). The results were then corrected to the urinary concentration of creatinine, which was determined with the Accuras Auto CRE (Shino-test Co.). The urinary biopyrrins/creatinine ratio was used in subsequent analyses (12). The intra-assay coefficient of variation for the same samples (n = 10) was 3.4% in triplicate, whereas the interassay coefficient of variation for the same samples (n = 10) was 8.5% in three experiments.

Plasma BNP assays. To determine plasma BNP, blood samples from an antecubital vein were obtained immediately after admission in all subjects. Blood samples for plasma BNP and urine samples for biopyrrins were obtained on the same day. The plasma BNP concentration was measured with a specific radioimmunoassay for human BNP, as previously described (14).

Statistical analysis. All results without plasma levels of BNP and urinary levels of biopyrrins/creatinine are expressed as the mean value ± SEM. Comparisons of continuous data between multiple groups were determined by one-way analysis of variance, followed by the Scheffé F test. The frequency data were compared by the chi-square test. Plasma levels of BNP and urinary levels of biopyrrins/creatinine were not distributed normally. Thus, the results of plasma BNP and urinary biopyrrins/creatinine levels are expressed as the median value (25th to 75th percentile range), and nonparametric analysis was used. The Mann-Whitney *U* test was used to evaluate differences in the levels of biopyrrins/creatinine between the two groups. Comparisons of plasma BNP and urinary biopyrrins/creatinine levels among multiple groups were determined by both the Kruskal-Wallis and Mann-Whitney *U* tests. Log BNP and log biopyrrins/creatinine levels were used for linear regression analysis, which was used to determine the correlation between the two variables. The changes in NYHA functional class before and after treatment were compared by the paired *t* test. Change in the levels of biopyrrins/creatinine were compared by the Wilcoxon signed-rank test. A p value <0.05 was considered significant.

RESULTS

The 94 patients with heart disease were classified into three classes according to NYHA functional classification: 35 patients were in NYHA class I, 33 in class II, and 26 in class III/IV.

The clinical characteristics of the study subjects are

Table 1. Patient Characteristics

	Control Group (n = 47)	Heart Failure Group (n = 94)
Age (yrs)	65 ± 2	65 ± 1
Men/women (n)	30/17	59/35
Hypertension	22 (47%)	46 (49%)
Smoking	9 (19%)	23 (24%)
Diabetes mellitus	7 (15%)	18 (19%)
Obesity (BMI ≥25 kg/m^2)	12 (26%)	25 (27%)
Total cholesterol (mg/dl)	192 ± 6	187 ± 3
HDL cholesterol (mg/dl)	51 ± 2	53 ± 2
LDL cholesterol (mg/dl)	121 ± 5	115 ± 3
Triglycerides (mg/dl)	133 ± 10	116 ± 8
Urinary biopyrrins/creatinine (μmol/g creatinine)	2.38 (1.57–3.15)	5.91 (3.33–10.45)*

*$p < 0.001$ vs. controls. Data are expressed as the mean value ± SEM or number (%) of subjects. Results of urinary biopyrrins/creatinine levels are expressed as the median value (25th to 75th percentile range).

BMI = body mass index; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

Table 2. New York Heart Association Functional Classes and Hemodynamic Variables

	Control Group (n = 47)	Class I (n = 35)	Class II (n = 33)	Class III/IV (n = 26)
HR (beats/min)	65 ± 2*†	70 ± 2*	77 ± 4*	98 ± 4
BP (mm Hg)	132 ± 2/78 ± 1	128 ± 4/75 ± 2	130 ± 6/72 ± 3	128 ± 7/81 ± 5
LVEF (%)	61.7 ± 1.4*‡	54.8 ± 2.7*	46.7 ± 2.7¶	36.3 ± 3.6
BNP (pg/ml)	24.9*‡§ (13.3–38.3)	80.2*‡ (29.3–153.0)	232.0* (109.1–294.0)	404.5 (280.0–686.0)

*$p < 0.001$ vs. class III/IV. †$p < 0.05$ vs. class II. ‡$p < 0.001$ vs. class II. §$p < 0.001$ vs. class I. ¶$p < 0.05$ vs. class III/IV. Data are expressed as the mean value ± SEM. Results of plasma BNP levels are expressed as the median value (25th to 75th percentile range).
BNP = B-type natriuretic peptide; BP = blood pressure; HR = heart rate; LVEF = left ventricular ejection fraction.

shown in Table 1. Heart rate was the highest in patients in NYHA class III/IV (Table 2). The LVEF was significantly lower in patients in NYHA classes II and III/IV than in controls (class II vs. controls: $p < 0.001$; class III/IV vs. controls: $p < 0.001$). The LVEF was reduced in patients in NYHA class III/IV, compared with patients in class I or II (class III/IV vs. class I: $p < 0.001$; class III/IV vs. class II: $p < 0.05$). There were no differences in LVEF between the controls and patients in NYHA class I. The BNP levels were the highest in patients in NYHA class III/IV. The plasma levels of BNP were significantly higher in patients in NYHA class I or II than in controls ($p < 0.001$). Moreover, each group had significant differences in plasma levels of BNP.

Urinary levels of biopyrrins/creatinine. The urinary levels of biopyrrins/creatinine (μmol/g creatinine) were significantly increased in patients with HF compared with controls ($p < 0.001$) (Table 1). The urinary levels of biopyrrins/creatinine were the highest in patients in NYHA class III/IV (17.05 [range 7.85 to 42.91]) (Fig. 1). The urinary levels of biopyrrins/creatinine in patients in NYHA class I (3.46 [range 2.60 to 5.42]) or II (5.39 [range 3.37 to 9.36]) were higher than those in controls (2.38 [range 1.57 to 3.15]) (Fig. 1). Furthermore, there were significant differences in urinary levels of biopyrrins/creatinine among the groups (Fig. 1). Log biopyrrins/creatinine levels were significantly and positively correlated with log BNP levels, as shown in Figure 2 ($r = 0.650$, $p < 0.001$).

Log biopyrrins/creatinine levels were significantly and positively correlated with pulmonary artery wedge pressure ($r = 0.327$, $p < 0.001$) and mean pulmonary artery pressure ($r = 0.389$, $p < 0.001$). Log biopyrrins/creatinine levels were significantly and negatively correlated with the cardiac index ($r = -0.338$, $p < 0.001$). However, there was no correlation between log biopyrrins/creatinine levels and right atrial pressure. Furthermore, log biopyrrins/creatinine levels were significantly and negatively correlated with LVEF ($r = -0.415$, $p < 0.001$). The treatment of HF decreased both the NYHA class (from 2.5 ± 0.1 to 1.7 ± 0.1, $p < 0.001$) and the biopyrrins/creatinine levels (from 7.43 [range 3.84 to 17.05] to 3.07 [range 2.21 to 5.71], $p < 0.001$), as shown in Figure 3.

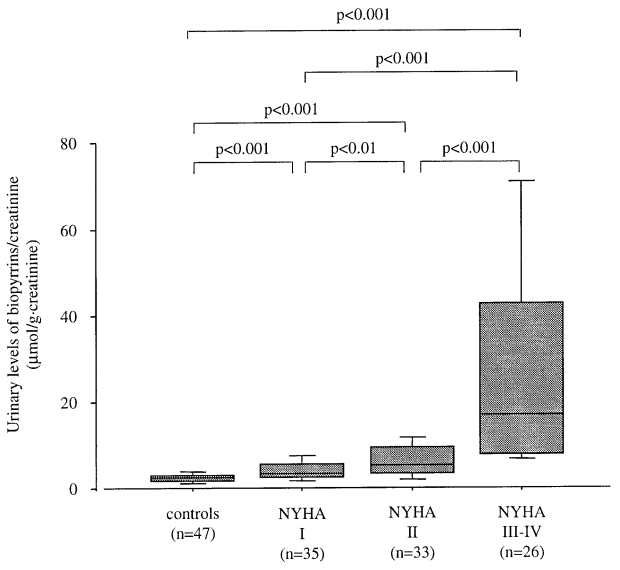

Figure 1. Comparison of urinary levels of biopyrrins/creatinine among the patients in New York Heart Association (NYHA) functional classes I, II, and III/IV and controls. The **horizontal line in the box** represents the median value; the **boxed area** is the interquartile range; and the **whiskers** are the 10% to 90% range.

DISCUSSION

To the best of our knowledge, this is the first clinical report to analyze the urinary levels of biopyrrins in patients with HF. The present study provides clinical evidence that the urinary levels of biopyrrins/creatinine are changed in association with both NYHA functional class and plasma levels of BNP.

Recently, increases in plasma biochemical markers of oxidative stress have been reported in patients with HF

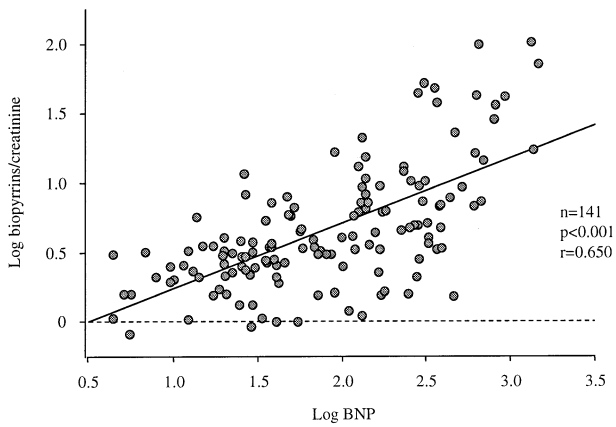

Figure 2. Correlation between log biopyrrins/creatinine levels and log B-type natriuretic peptide (BNP) levels.

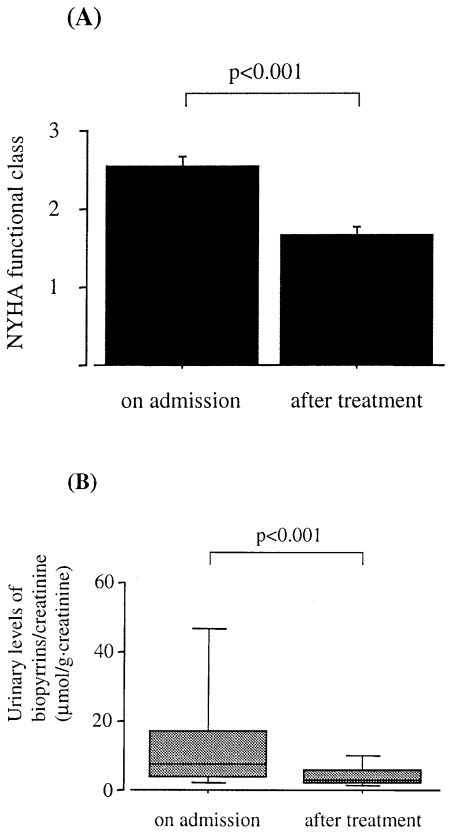

Figure 3. (A) Bar graphs comparing New York Heart Association (NYHA) functional classes before and after the active treatment of heart failure (mean ± SEM). **(B)** Box plots of urinary biopyrrins/creatinine levels before and after the active treatment of heart failure. The **horizontal line in the box** represents the median value; the **boxed area** is the interquartile range; and the **whiskers** are the 10% to 90% range.

(17,18). There is a definitive correlation between oxidative stress and ventricular dysfunction (2,4). Furthermore, ventricular remodeling and progressive dilation leading to end-stage HF may be mediated by oxygen-derived free radicals (2,4). Therefore, it is likely that ROS are involved in not only the pathogenesis but also the active progression of HF (3–6). In an attempt to prevent the production of oxidants, as well as to ameliorate and repair oxidative tissue damage, detoxification systems are present in vivo and comprise both enzymatic and nonenzymatic antioxidant compounds. Stocker et al. (7,19) have demonstrated a beneficial role for bilirubin by demonstrating the powerful antioxidant activity of bilirubin in vivo. The antioxidant effect of bilirubin exceeded that of alpha-tocopherol under 2% oxygen, which is near physiologic intracellular oxygen levels (20). In the present study, we measured biopyrrins, the oxidative metabolites of bilirubin, in patients with HF. We provide clinical evidence that the urinary levels of biopyrrins/creatinine are significantly increased in patients with HF compared with controls. These data are compatible with the finding that HF is associated with increased oxidative stress, the extent of which has a close relation to the degree of HF (17,21,22). Furthermore, the present study demonstrates that the urinary levels of biopyrrins in patients with HF were elevated in proportion to its severity and subsequently decreased after the active treatment of HF. Thus, we postulate that the increased urinary biopyrrins levels may indirectly reflect the excessive oxidative stress caused by ROS associated with uncontrolled HF, and that there is an apparent normalization of these indexes of oxidative stress after treatment of HF.

The etiology of increased oxidative stress is still unclear. Heart failure is a clinical syndrome characterized by long-term, persistent activation of the sympathetic nervous system and renin-angiotensin-aldosterone system (23,24). The homeostatic mechanisms seem activated in response to a perceived reduction in circulating blood volume in patients with HF. The resultant effect is the development of a vicious cycle characterized by excessive neurohormonal stimulation that is responsible for not only the persistent expression of adverse hemodynamic abnormalities but also myocardial and vascular remodeling, a hallmark of progressive HF (23). For example, norepinephrine is elevated in HF, because sympathetic activation is an early mechanism that maintains cardiac output in HF (23,25). However, norepinephrine produces free radicals and promotes cardiomyocyte apoptosis (25–27). The loss of cardiomyocytes by apoptosis has emerged as an important factor contributing to ventricular remodeling (25). The cardiomyocyte apoptosis and generation of ROS may be triggered by mechanical force, cytokines (e.g., angiotensin II), and neurotransmitters (e.g., norepinephrine) (25). Moreover, many studies have demonstrated that oxidative stress may activate the apoptotic cell death of cardiomyocytes (25,28). Thus, oxidative stress contributes to ventricular remodeling, and the magnitude of oxidative stress is related to the severity of HF.

On the other hand, plasma levels of BNP reflect the degree of left ventricular remodeling, damage, or dysfunction, and BNP is an important prognostic predictor for patients with HF (29–32). In the present study, to examine whether the urinary biopyrrins levels in patients with HF might be related to the severity of HF, we also examined the plasma levels of BNP in addition to NYHA functional class. The present study showed that log biopyrrins/creatinine levels are closely and positively correlated to log BNP levels. Natriuretic peptides are released in response to increased intracardiac volume or pressure (33). It has been shown that overstretching of the myocardium leads to enhanced generation of ROS (34). Moreover, angiotensin II may be considered a prime stimulus of oxidative stress and increases generation of ROS within cardiomyocytes (35). Production of ROS may cause mitochondrial DNA damage and decrease activity, thereby contributing to the increase in oxidative stress. Furthermore, angiotensin II also worsens HF. In rats, infusion of angiotensin II induced expression of various genes, including BNP (36). Suo et al. (37) demonstrated that left ventricular BNP messenger ribonucleic acid and immunoreactive BNP levels are distinctly regulated by BNP promoter activity in an angiotensin II–induced model

of experimental hypertension in vivo. These pathophysiologic mechanisms might account for the significant correlation between the urinary levels of biopyrrins and plasma levels of BNP in patients with HF. In fact, both the NYHA functional class and the biopyrrins/creatinine levels decreased after treatment of HF. In congestive HF, myocardial contractility is impaired by either a loss of muscle or by pressure or volume overload, which causes myocardial ischemia and generation of ROS (38,39). Based on these results, we postulated that urinary biopyrrins levels, a new oxidative stress marker, related to the severity of HF and had a close correlation with BNP.

In the present study, the ejection fractions from each of the categories of HF patients are high. In previous study, it was demonstrated that plasma BNP levels increase in patients with diastolic dysfunction (40). Because we used BNP as one of the parameters in HF severity, the causes of HF included not only systolic dysfunction but also diastolic dysfunction. Moreover, the patients with active ischemia causing intermittent cardiac congestion comprised 6% of the HF group. These may be reasons that the ejection fractions from each of the categories of HF patients are high.

Study limitations. In the present study, antioxidants (e.g., vitamin E, carvedilol) did not have any effects on the biopyrrins levels. Unfortunately, the observation period may be too short, and the antioxidant therapy group may be too small. In addition, we excluded both patients with renal failure and those with liver dysfunction. We need further studies to clarify the effects of antioxidants and liver and renal diseases on biopyrrins levels.

We do not have detailed data on the half-life of biopyrrins. We reported dynamic changes of biopyrrins within a few hours in patients with acute myocardial infarction (12). Other investigators also showed dynamic changes of biopyrrins within 24 h after acute coronary artery occlusion in patients with coronary spasm (41). These data suggest that the concentration of biopyrrins might change within a short time in humans.

Conclusions. The urinary levels of biopyrrins/creatinine are closely related to both NYHA functional class and plasma levels of BNP. Because the measurement of urinary biopyrrins is noninvasive, urinary biopyrrins may be clinically useful markers of oxidative stress. The urinary biopyrrins/creatinine levels may imply the HF severity.

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